AAC
Journals.ASM.org

Effect of Linezolid on the 50% Lethal Dose and 50% Protective Dose in Treatment of Infections by Gram-Negative Pathogens in Naive and Immunosuppressed Mice and on the Efficacy of Ciprofloxacin in an Acute Murine Model of Septicemia

Andrea Marra,[a]* Lucinda Lamb,[a]* Ivette Medina,[a]* David George,[b] Glenn Gibson,[a]* Joel Hardink,[c] Jady Rugg,[c] Jeffrey Van Deusen,[c] and John P. O'Donnell*[a]

Pfizer Worldwide Research and Development, Groton, Connecticut, USA[a]; Antibacterials Research Unit, Pfizer Worldwide Research and Development, Groton, Connecticut, USA[b]; and Department of Pharmacokinetics, Dynamics and Metabolism, Pfizer Worldwide Research and Development, Groton, Connecticut, USA[c]

Murine models of infection were used to study the effect of linezolid on the virulence of Gram-negative bacteria and to assess potential pharmacodynamic interactions with ciprofloxacin in the treatment of these infections, prompted by observations from a recent clinical trial. Naive and immunosuppressed mice were challenged with *Klebsiella pneumoniae* 53A1109, *K. pneumoniae* GC6658, and *Pseudomonas aeruginosa* UC12120 in acute sepsis and pulmonary infection models, using different serial dilutions of these pathogens (groups of 8 animals each). Linezolid (100 mg/kg/dose) was administered orally at 0.5 and 4.0 h postchallenge in the sepsis model and at 4 h postchallenge followed by 2 days of twice-daily treatment in the pulmonary model. Further, ciprofloxacin alone and in combination with oral linezolid was investigated in the sepsis model. Survival was assessed for 4 and 10 days postchallenge in the systemic and respiratory models, respectively. The data were fitted to a nonlinear regression analysis to determine 50% lethal doses (LD_{50}s) and 50% protective doses (PD_{50}s). A clinically relevant, high-dose regimen of linezolid had no significant effect on LD_{50} in these models. This lack of effect was independent of immune status. A combination of oral ciprofloxacin with linezolid yielded lower PD_{50}s than oral ciprofloxacin alone (ciprofloxacin in combination, 8.4 to 32.7 mg/kg; oral ciprofloxacin, 39.4 to 88.3 mg/kg). Linezolid did not improve the efficacy of subcutaneous ciprofloxacin (ciprofloxacin in combination, 2.0 to 2.4 mg/kg; subcutaneous ciprofloxacin, 2.0 to 2.8 mg/kg). In conclusion, linezolid does not seem to potentiate infections caused by Gram-negative pathogens or to interact antagonistically with ciprofloxacin.

Linezolid, an antibacterial agent of the oxazolidinone class, has demonstrated broad activity against many clinically important Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus*, coagulase-negative staphylococci, vancomycin-resistant enterococci, penicillin-resistant *Streptococcus pneumoniae*, viridans group streptococci, and various serotypes of beta-hemolytic streptococci, and against more rarely isolated pathogens (2, 4, 5, 10). Linezolid is ineffective against most aerobic Gram-negative bacteria, which necessitates combination therapy if a concomitant Gram-negative pathogen is documented or suspected.

A recent open-label, multicenter, comparative, phase 3 clinical trial showed linezolid to be noninferior to vancomycin in patients presenting with complicated skin and skin structure infections and catheter-related bloodstream infections due to Gram-positive pathogens (22). However, a mortality imbalance (linezolid group, 21.5%; control group, 16.0%) was observed at 12 weeks posttreatment in the overall intent-to-treat population of that study. Kaplan-Meier survival curves revealed that much of this imbalance occurred in patients with Gram-negative pathogens or those who had negative culture results at baseline, rather than the primary analysis population. Notably, less than half of patients with infections caused by Gram-negative organisms in both the linezolid and control groups appeared to have received adequate antibiotic therapy (defined as ≥1 antibiotic active against the organism within 24 h of culture) for these infections (22). Furthermore, the authors found no evidence that the mortality imbalance could be attributed to adverse effects of linezolid therapy, such as effects on cytokine production or neutrophil function, or antagonism (22). However, a specific reason for this imbalance could not be determined with certainty, due to the *post hoc* nature of these analyses.

The objective of the present study was to determine whether administration of linezolid at clinically relevant concentrations would increase or alter the virulence of representative infections caused by the Gram-negative pathogens *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, using murine models of septicemia and pulmonary infections. An additional aim of the investigation was to explore whether linezolid adversely affected the 50% protective dose (PD_{50}) and bacterial killing of concomitant Gram-negative therapy with the fluoroquinolone ciprofloxacin, considering that patients with mixed infections with Gram-negative and Gram-positive organisms are often treated with a combination of

Received 3 February 2012 Returned for modification 29 March 2012
Accepted 2 June 2012

Published ahead of print 18 June 2012

Address correspondence to John P. O'Donnell, john.odonnell@astrazeneca.com.

* Present address: Andrea Marra, Rib-X Pharmaceuticals, New Haven, Connecticut, USA; Lucinda Lamb, Center for Anti-infective Research and Development, Hartford Hospital, Hartford, Connecticut, USA; Ivette Medina, Clinical RM, Hinckley, Ohio, USA; Glenn Gibson, Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut, USA; John P. O'Donnell, Department of DMPK, AstraZeneca Pharmaceuticals LP, Waltham, Massachusetts, USA.

doi:10.1128/AAC.00276-12

antibacterial agents. The possibility of a pharmacokinetic interaction between linezolid and ciprofloxacin in infected mice was also investigated.

MATERIALS AND METHODS

Bacterial strains. Three clinical Gram-negative isolates were used in these *in vivo* studies. *K. pneumoniae* 53A1109 is an extended-spectrum ß-lactamase (ESBL)-positive isolate possessing TEM-1, OXA-9, multiple SHVs, and AmpC ß-lactamase genes. This organism is resistant to amikacin, aztreonam, many cephalosporins, and β-lactam antibiotics. *K. pneumoniae* GC6658 is a penicillin- and macrolide-resistant ESBL-positive isolate. *P. aeruginosa* UC12120 is a fluoroquinolone-sensitive strain.

All organisms were obtained from the Pfizer bacterial culture collection. Confirmation of bacterial identification and generation of initial antibiograms was conducted using a BD Phoenix automated microbiology system (BD Diagnostics, Sparks, MD). MIC testing of the *K. pneumoniae* and *P. aeruginosa* strains was conducted against ceftazidime, ciprofloxacin, imipenem, and linezolid according to Clinical and Laboratory Standards Institute guidelines (3). Stock solutions used for susceptibility testing were prepared in 3% dimethyl sulfoxide in water immediately prior to use.

Antimicrobial agents. The antimicrobials linezolid (Zyvox tablets; lot 85HKT; Pharmacia & Upjohn Co.), ciprofloxacin (tablets; lot 5CAR; Miles Inc.), imipenem/cilastatin (Primaxin sterile lyophile; lot 3681M; Merck and Co.), levofloxacin (lot 446423/1; Fluka Biochemika), and ceftazidime (Fortaz sterile lyophile; lot 6ZP0908; Glaxo Pharmaceuticals) were obtained commercially. Oral formulations of linezolid and ciprofloxacin were prepared in 10% ethanol–90% methylcellulose (0.5%) and sterile water, respectively. Ciprofloxacin, imipenem, and ceftazidime were reconstituted in sterile water for subcutaneous administration. Dilution of compounds into dosing vehicles was completed immediately prior to administration.

Animal studies. All procedures involving animals were conducted in accordance with guidelines established by the Pfizer Institutional Animal Care and Use Committee. Experiments to determine the effect of linezolid on the 50% lethal dose (LD_{50}) of *K. pneumoniae* 53A1109, *K. pneumoniae* GC6658, and *P. aeruginosa* UC12120 were conducted in both naive and immunosuppressed mice. Animals were housed in groups of 5 to 10 mice per cage and given access to food and water *ad libitum* for all studies. For immunosuppression studies, animals were rendered transiently neutropenic by two oral doses of cyclophosphamide monohydrate (Sigma-Aldrich, St. Louis, MO). The first dose (150 mg/kg in 0.2 ml sterile water) was administered 4 days prior to infection, and the second dose (100 mg/kg in 0.2 ml sterile water) was administered 1 day prior to infection.

Acute septicemia model. Groups of 8 CF-1 female mice (Charles River Laboratories) were infected intraperitoneally with serial 10-fold dilutions of *K. pneumoniae* 53A1109 or *K. pneumoniae* GC6658 in 3% brewer's yeast (Sigma-Aldrich, St. Louis, MO). Inocula were prepared from frozen stock with 100 μl spread on a 5% sheep blood agar plate and incubated overnight at 37°C. The plate was washed with brain heart infusion broth (BHIB) and diluted to a targeted inoculum per mouse to be delivered in 0.5 ml. For LD_{50} studies, conducted as two separate experiments (see Table 1 for details), the inocula ranged from about 0.1 to 9.2 $\log_{10}$ CFU/group, depending on strain and immune status. For each pathogen and at each inoculum, the LD_{50} was determined in both naive and immunosuppressed mice with and without linezolid treatment (dosed at 100 mg/kg at 0.5 and 4.0 h postinfection, yielding a total dose of 200 mg/kg). For the PD_{50} studies, which were conducted as 4 separate experiments, inocula ranged from about 1.15×10^7 to 2×10^7 CFU/group (see Table 2 for details). PD_{50} was determined for ciprofloxacin administered orally (PO) and subcutaneously (SC), both alone and with concomitant PO linezolid (dosed at 100 mg/kg). Ciprofloxacin was administered at 4 doses: 1.56, 6.25, 25, and 100 mg/kg for PO ciprofloxacin and 0.78, 3.12, 12.5, and 50 mg/kg for SC ciprofloxacin. The PD_{50} was also determined for SC imipenem (at 0.30, 1.25, 5, and 20 mg/kg) and SC ceftazidime (50 mg/kg) as control agents. For the PD_{50} experiments, all agents were administered at 0.5 and 4 h postinfection. In all experiments, animal survivorship was assessed for 4 days following bacterial challenge, and LD_{50}s and PD_{50}s were determined from nonlinear regression analysis of the data using GraphPad Prism version 3.02 (GraphPad Software, La Jolla, CA).

Pulmonary infection model. Groups consisting of 8 isoflurane-anesthetized C3H/HeN female mice (Charles River Laboratories) were infected intranasally with serial 10-fold dilutions of *P. aeruginosa* UC12120 by placing 40 μl of bacterial suspension in BHIB onto the external nares.

TABLE 1 LD_{50}s for *K. pneumoniae* 53A1109, *K. pneumoniae* GC6658, and *P. aeruginosa* UC12120 in naive and immunosuppressed mice with and without linezolid treatment

Infection and mouse group	Treatment	LD_{50} ($\log_{10}$ CFU/mouse) (95% CI)[a]	
		Expt 1	Expt 2
Acute septicemia with *K. pneumoniae* 53A1109			
Naïve	None	5.1 (3.4–6.7)[b]	5.0 (3.4–6.6)[c]
Naïve	Linezolid		5.8 (3.9–7.7)[c]
Immunosuppressed	None	<1.7[d]	<1.0[e]
Immunosuppressed	Linezolid	<1.7[d]	1.1 (1.0–1.1)[e]
Acute septicemia with *K. pneumoniae* GC6658			
Naïve	None	5.2 (3.7–6.6)[f]	6.3 (4.3–8.3)[g]
Naïve	Linezolid	7.0 (4.6–9.3)[f]	6.2 (4.3–8.2)[g]
Immunosuppressed	None	<2.2[h]	<0.1[i]
Immunosuppressed	Linezolid	<2.2[h]	0.7 (−0.4–1.8)[i]
Pulmonary infection with *P. aeruginosa* UC12120			
Naïve	None	7.7 (3.2–12)[j]	6.8 (1.8–12)[k]
Naïve	Linezolid		
Immunosuppressed	None	1.1 (0.6–1.6)[l]	2.2 (0.7–3.8)[m]
Immunosuppressed	Linezolid	1.4 (1.3–1.5)[l]	1.9 (1.1–2.8)[m]

[a] Determined from groups of mice ($n = 8$ each), with each group receiving a different bacterial infection dose (increasing 10-fold from group to group); experiments were not duplicates. The 95% CIs were parametric.
[b] Determined from 6 groups of mice, with infection doses ranging from 2.74 to 7.74 $\log_{10}$ CFU.
[c] Determined from 6 groups of mice, with infection doses ranging from 3.04 to 8.04 $\log_{10}$ CFU.
[d] Determined from 6 groups of mice, with infection doses ranging from 1.74 to 6.74 $\log_{10}$ CFU.
[e] Determined from 6 groups of mice, with infection doses ranging from 1.04 to 6.04 $\log_{10}$ CFU.
[f] Determined from 6 groups of mice, with infection doses ranging from 4.15 to 9.15 $\log_{10}$ CFU.
[g] Determined from 6 groups of mice, with infection doses ranging from 4.10 to 9.10 $\log_{10}$ CFU.
[h] Determined from 6 groups of mice, with infection doses ranging from 2.15 to 7.15 $\log_{10}$ CFU.
[i] Determined from 6 groups of mice, with infection doses ranging from 0.10 to 5.10 $\log_{10}$ CFU.
[j] Determined from 4 groups of mice, with infection doses ranging from 4.1 to 7.1 $\log_{10}$ CFU.
[k] Determined from 4 groups of mice, with infection doses ranging from 3.9 to 6.9 $\log_{10}$ CFU.
[l] Determined from 4 groups of mice, with infection doses ranging from 0.1 to 3.1 $\log_{10}$ CFU.
[m] Determined from 4 groups of mice, with infection doses ranging from <0.1 to 2.9 $\log_{10}$ CFU.

TABLE 2 PD_{50}s of ciprofloxacin, linezolid, and ciprofloxacin in combination with linezolid against *K. pneumoniae* 53A1109 in a murine model of acute septicemia

Compound	Route	PD_{50} (mg/kg) (95% CI) in expt:			
		1[a]	2[b]	3[b]	4[c]
Ciprofloxacin[d]	PO	39.4 (36–43)	88.3 (69–110)	63.4 (59–68)	77.0 (76–79)
Ciprofloxacin[d] + linezolid	PO/PO	19.1 (14–24)	22.1 (15–29)	8.4 (0.0–19)	32.7 (24–42)
Linezolid[e]	PO	>100	>100	>100	>100
Ciprofloxacin[f]	SC			2.0 (1.8–2.1)	2.8 (2.7–2.8)
Ciprofloxacin[f] + linezolid	SC/PO			2.4 (0.82–3.9)	2.0 (1.0–2.9)
Imipenem/cilastatin	SC	1.1 (1.1–1.1)	5.6 (−2.2–13.4)	0.79 (0.74–0.84)	1.41 (1.40–1.43)
Ceftazidime	SC	>50	>50	>50	>50

[a] Inoculum of 2×10^7 CFU.
[b] Inoculum of 1.15×10^7 CFU.
[c] Inoculum of 1.5×10^7 CFU.
[d] Each PD_{50} was determined from 4 groups of mice ($n = 8$ per group); each group received a different ciprofloxacin dose: 1.56, 6.25, 25, or 100 mg/kg.
[e] Each PD_{50} was determined from a single group of mice ($n = 8$).
[f] Each PD_{50} was determined from 4 groups of mice ($n = 8$ per group); each group received a different ciprofloxacin dose: 0.78, 3.12, 12.5, or 50 mg/kg.

Each mouse was held in a vertical position until the droplet was completely inhaled. Inocula ranged from 0.1 to 7.1 $\log_{10}$ CFU/group (see Table 1 for details). The LD_{50} was determined in naive mice without any treatment and in immunosuppressed mice with and without linezolid treatment. Linezolid was administered PO beginning at 4 h postinfection and subsequently for 2 days of twice-daily therapy (i.e., at 24, 32, 48, and 56 h postinfection) for a total of five doses. Animal survival was assessed for 10 days following bacterial challenge, and LD_{50}s were determined from nonlinear regression analysis of the data using GraphPad Prism version 3.02.

Pharmacokinetic studies. CF-1 mice were infected intraperitoneally with *K. pneumoniae* 53A1109 and at 0.5 and 4.0 h postinfection were given either ciprofloxacin alone (50 mg/kg by oral gavage), linezolid alone (100 mg/kg by oral gavage), or both ciprofloxacin and linezolid concurrently (50 mg/kg and 100 mg/kg, respectively, by oral gavage). Samples from terminal bleeds were obtained at 0.25, 0.5, 1, 2, 4, 6, and 12 h after the initial dose (5 mice/time point). Plasma was analyzed for ciprofloxacin and linezolid concentrations using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Briefly, plasma samples were subject to protein precipitation with acetonitrile containing an internal standard (levofloxacin), and 5 μl of the resulting supernatant was injected onto a MAC MOD Halo C_{18} column (2.7 μm, 30 by 3 mm; MAC-MOD Analytical, Chadds Ford, PA). The column was equilibrated with mobile phase (A, 5 mM ammonium formate with 0.05% formic acid; B, acetonitrile-mobile phase A [80:20]) at a flow rate of 1.2 ml/min. The gradient was started at 5% B and was increased to 80% B from 0.3 to 1.4 min. Conditions were held at 80% B until 1.6 min, were returned to starting conditions by 1.7 min, and were held for an additional 0.4 min, for a total run time of 2.1 min. Under these conditions, the retention times for ciprofloxacin, linezolid, and the internal standard, levofloxacin, were 0.74, 0.84, and 0.73 min, respectively. The effluent was analyzed with an API-4000 mass spectrometer detector (AB Sciex, Foster City, CA), fitted with a turbo ion spray interface and operated in positive-ion mode. The declustering potential, collision energy, and collision cell exit potential, respectively, were 72, 26, and 15 for ciprofloxacin, 46, 27, and 18 for linezolid, and 70, 30, and 20 for levofloxacin at a temperature of 600°C. Ciprofloxacin, linezolid, and levofloxacin were monitored by multiple reactions monitoring at transitions of 332.0/288.2, 338.3/296.3, and 362.3/318.0, respectively. The dynamic range of the assay was 0.0025 to 10 μg/ml for ciprofloxacin and 0.005 to 10 μg/ml for linezolid.

Pharmacokinetic analysis. Linezolid and ciprofloxacin concentrations (mean data for 5 mice per time point were used to generate a single concentration-time curve) were fitted to a 1- or 2-compartment model using WinNonLin version 5.2 (Pharsight, St. Louis, MO). Maximum concentration (C_{max}) was the highest observed concentration; T_{max} was the earliest time at which C_{max} occurred; and the elimination half-life was estimated as $\ln 2/k_{el}$, where k_{el} is the elimination rate constant derived from the slope of the log concentration-versus-time profile. The area under the concentration-time curve from 0 h to the last time point (AUC_{0-12}) was calculated by linear trapezoidal approximation.

RESULTS

Effect of linezolid on the virulence (LD_{50}) of *K. pneumoniae* and *P. aeruginosa*. LD_{50}s expressed in terms of bacterial challenge ($\log_{10}$ CFU/mouse) with *K. pneumoniae* 53A1109, *K. pneumoniae* GC6658, and *P. aeruginosa* UC12120 are summarized in Table 1. As expected, inoculum LD_{50}s were consistently higher in naive animals than in those rendered neutropenic. Administration of 100 mg/kg linezolid per dose had no effect on the virulence of any of the three Gram-negative strains evaluated, as evident from the observation that 95% confidence intervals (CIs) overlapped with those from non-drug-treated animals. This was the case for immunocompetent as well as immunosuppressed mice.

Efficacy (PD_{50}) of ciprofloxacin, linezolid, and the combination of ciprofloxacin and linezolid against *K. pneumoniae* 53A1109 in a model of acute septicemia. The PD_{50}s of ciprofloxacin (MIC = 0.5 μg/ml), linezolid (MIC > 256 μg/ml), and ciprofloxacin dosed in combination with linezolid are presented in Table 2. PD_{50}s for linezolid were >100 mg/kg in all experiments, and mean PD_{50}s for ciprofloxacin were 67 and 2.4 mg/kg following PO and SC administration, respectively. PD_{50}s for ciprofloxacin following SC administration were approximately 30-fold lower than values reported after PO dosing in the same experiments. Coadministration of linezolid resulted in significantly lower oral PD_{50}s for PO ciprofloxacin (as evident from the respective nonoverlapping 95% CIs). No changes in cytokine levels were observed between monotherapy and the combination (data not shown). Comparator control drugs, imipenem and ceftazidime, demonstrated consistent PD_{50}s of 0.8 to 5.6 mg/kg and >50 mg/kg, respectively, across all 4 experiments.

Pharmacokinetics of ciprofloxacin and linezolid in infected mice. Pharmacokinetics of ciprofloxacin and linezolid PO administration are summarized in Table 3. Exposure of ciprofloxacin following PO administration of 50 mg/kg doses at 0.5 and 4 h postinfection demonstrated a C_{max} of <1 μg/ml. The plasma concentration over time of ciprofloxacin alone compared to ciprofloxacin coadministered with linezolid is shown in Fig. 1. Overall, linezolid appeared to have little effect on the pharmacokinetics of ciprofloxacin.

TABLE 3 Pharmacokinetics of ciprofloxacin and linezolid in infected mice following twice-daily PO administration[a]

Compound (dose [mg/kg]×)	T_{max} (h)	C_{max} (μg/ml)	AUC_{0-12} (μg · h/ml)
Ciprofloxacin (50)	12.0	0.93	8.11
Ciprofloxacin (50), in combination	6.0	1.32	9.16
Linezolid (100)	6.0	38.0	355.0
Linezolid (100), in combination	6.0	46.8	362.8

[a] Each treatment group consisted of 35 mice; animals were sacrificed at 0.25, 0.5, 1, 2, 4, 6, or 12 h postdose (5 mice per time point). The resulting mean data were used to generate a single concentration-versus-time profile for each treatment group, and the resulting pharmacokinetic parameters were calculated from that profile; hence, no statistical comparisons are shown.

DISCUSSION

Since combination therapy is often needed for the effective treatment of a potential mixed bacterial infection, careful consideration of appropriate agents to provide optimum treatment is of utmost importance. Whereas synergistic combinations are ideal in a setting where pathogens are known, it seems even more critical that combinations of agents employed to ensure broad-spectrum coverage in empirical therapy do not result in antagonism.

Since the major spectrum limitation for linezolid is a lack of Gram-negative activity, it is important to establish that the use of ß-lactams with Gram-negative activity, quinolones, or other appropriate Gram-negative antibacterial agents is unlikely to result in antagonism when used in combination therapy with linezolid against a potential mixed infection. Using fractional inhibitory concentration (FIC) indices and a checkerboard approach, a comprehensive *in vitro* investigation that studied the activity of linezolid in combination with 35 other antimicrobial agents against Gram-positive and Gram-negative organisms revealed a mostly indifferent response (1,369 out of 1,380 linezolid-drug combinations) (20). Linezolid plus amoxicillin resulted in synergy against 3 strains of methicillin-resistant *S. aureus*. Low levels of antagonism were only observed with combinations using ofloxacin and sparfloxacin against *E. faecalis*. Although the FIC index derived using the checkerboard technique is both popular and simple to obtain (15), it has several well-documented limitations (9). A more recent *in vitro* study of linezolid, daptomycin, and vancomycin suggested there was some attenuation of activity against *Escherichia coli* ATCC 25922 when linezolid was used in combination with ceftazidime or aztreonam at 48 h in an *in vitro* pharmacodynamic model (IVPM) system (11). However, in a separate study, no antagonism was observed in an IVPM system using linezolid in combination with aztreonam or ceftazidime at clinically relevant concentrations against the same *E. coli* strain (6). Of note, these studies involved the use of dynamic as opposed to static drug concentrations and are therefore potentially subject to higher variability in pharmacodynamic response.

Extrapolation of these findings to the *in vivo* setting have been limited. A pharmacokinetic evaluation of linezolid and aztreonam showed that concomitant administration resulted in no clinically significant changes in drug distribution disposition relative to the pharmacokinetics established for each agent alone (17). In our investigation, we first set out to establish whether the administration of linezolid at or above clinically relevant concentrations resulted in a difference in virulence or pathogenesis caused by clinical isolates of *K. pneumoniae* and *P. aeruginosa* as assessed by an LD_{50} of bacterial density. In all the experiments described in this paper, linezolid was administered at 100 mg/kg/dose, a level that has previously been associated with preclinical efficacy in mouse models of infection (14, 23). In the present study, oral linezolid at this dose level had no significant effect on LD_{50}s in Gram-negative acute septicemia and pulmonary infection models. PD_{50} data for *K. pneumoniae* 53A1109 showed that linezolid alone was ineffective against this Gram-negative pathogen, with PD_{50}s in excess of 100 mg/kg. Pharmacokinetic analysis in mice at this dose level showed that the AUC_{0-12} for linezolid was approximately 360 μg · h/ml. Since protein binding of linezolid is uniformly low (about 30%) in humans (13, 16) and animals (18), the unbound AUC_{0-12} in mice would thus have been well in excess of that in healthy volunteers and patients given 600 mg PO twice daily (13, 16, 19) as well as in patients with severe sepsis/septic shock after a single 600-mg intravenous dose (21). Since linezolid did not adversely affect either the virulence of Gram-negative strains or the bacterial killing of ciprofloxacin at these exposures, it seems unlikely that this would happen at the lower exposures observed clinically. The ciprofloxacin AUC_{0-12} observed in these studies appears to be very comparable to levels seen in patients receiving PO ciprofloxacin at standard doses of 500 mg or 750 mg twice daily (1); the protein binding of ciprofloxacin in humans ranges from 20% to 40% (1, 7) and is around 38% in mice (12).

Consistent with previous *in vitro* checkerboard studies (6, 11, 20), there was no evidence of *in vivo* antagonism or synergy of the combination of linezolid and ciprofloxacin. These findings are of particular interest given the recent phase 3 comparison of linezolid and vancomycin in patients with complicated skin and skin structure infections and catheter-related bloodstream infections due to Gram-positive pathogens, in which a mortality imbalance was reported for the linezolid arm (22). The present data support the explanation put forward by the authors of that study, i.e., that the imbalance may have been attributable to a lack of adequate therapy for infections with Gram-negative organisms (22) or, alternatively, that it may even have been a chance finding. Our findings do not suggest that this mortality imbalance was due to suspected effects of linezolid therapy, such as increased virulence of key Gram-negative pathogens or changes in cytokine lev-

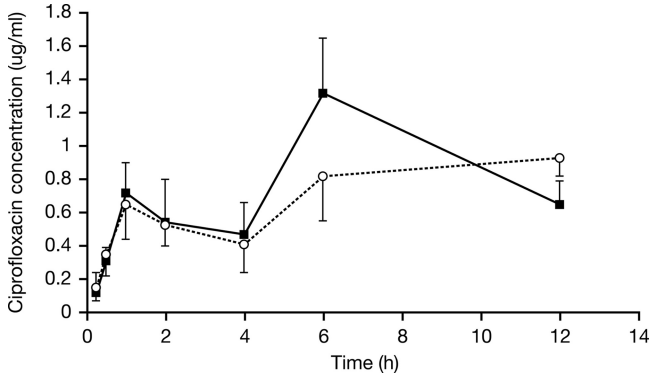

FIG 1 Mouse plasma time-versus-concentration profiles of ciprofloxacin following oral administration of 50 mg/kg alone (○) or in combination with 100 mg/kg of linezolid (■) at 0.5 and 4.0 h postinfection in a murine model of septicemia. Data are means ± standard deviations for 5 mice per time point.

els, or because of antagonism between linezolid and fluoroquinolones.

An interesting observation from the present study was that the PD_{50}s for SC ciprofloxacin were approximately 30-fold lower than the corresponding values for PO ciprofloxacin and were 10-fold lower on average with SC ciprofloxacin-linezolid versus PO ciprofloxacin-linezolid from the same set of experiments, suggestive of route-dependent activity. This is not a unique observation, since a 10-fold lower 50% effective dose has been reported previously with subcutaneous versus oral ciprofloxacin in an experimental systemic mouse model of *K. pneumoniae* infection (8). This disparity is not entirely understood, but can be partially reconciled with the relatively low oral bioavailability of ciprofloxacin in mice (ranging from 12% to 38%) (12; Pfizer, data on file), which is likely a key factor in the apparent administration route dependency of the PD_{50}. Differences in activity attributed to a change in immune response would be expected to be independent of the administration route and are unlikely to explain our results. Nevertheless, this apparent administration route dependency of the PD_{50} does not affect the conclusion derived from these experiments, since outcomes of monotherapy and combination therapy were compared within each specific administration route (thus eliminating the administration route effect).

In conclusion, linezolid, a compound lacking appreciable activity against Gram-negative organisms, did not increase the virulence of selected strains of *K. pneumoniae* and *P. aeruginosa* when administered in a clinically relevant, high-dose regimen, regardless of immune status. These results therefore suggest that linezolid did not potentiate infections caused by these bacteria. The data presented here also suggest that linezolid did not interact antagonistically with ciprofloxacin against *K. pneumoniae*. However, more work using *in vitro* dynamic model systems to further understand the combined activity of linezolid and ciprofloxacin against important Gram-negative pathogens is warranted.

ACKNOWLEDGMENTS

We thank Ping Liu (Pfizer Inc.) for her critical review and feedback. Susceptibility data were provided by Michael Huband.

This study was sponsored by Pfizer Inc. Editorial assistance was provided by Dominik Wolf at PAREXEL and was funded by Pfizer Inc.

All authors were employees of Pfizer Inc. at the time the studies were completed. L. Lamb, D. George, G. Gibson, J. Hardink, J. Rugg, J. Van Deusen, and J. P. O'Donnell were employees of Pfizer Inc. during the drafting of the manuscript.

REFERENCES

1. **Bayer Healthcare.** 2008. Cipro® (ciprofloxacin hydrochloride) tablets, Cipro® (ciprofloxacin) oral suspension. Revised October 2008. http://www.univgraph.com/bayer/inserts/ciprotab.pdf.
2. **Brickner SJ.** 1996. Oxazolidinone antibacterial agents. Curr. Pharm. Des. **2**:175–194.
3. **CLSI.** 2010. Performance standards for antimicrobial susceptibility testing; twentieth informational supplement. CLSI document M100–S20. CLSI, Wayne, PA.
4. **Diekema DJ, Jones RN.** 2001. Oxazolidinone antibiotics. Lancet **358**: 1975–1982.
5. **Farrell DJ, Mendes RE, Ross JE, Sader HS, Jones RN.** 2011. LEADER Program results for 2009: an activity and spectrum analysis of linezolid using 6,414 clinical isolates from 56 medical centers in the United States. Antimicrob. Agents Chemother. **55**:3684–3690.
6. **Hanna D, et al.** 2009. Pharmacokinetic/pharmacodynamic (PK/PD) analysis of linezolid (LZD) in combination with aztreonam (ATM) and ceftazidime (CAZ) against *E. coli*: use of *in vitro* PK models to assess combinations at therapeutically relevant drug concentrations, abstr A1-547. Abstr. 49th Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
7. **Höffken G, Lode H, Prinzing C, Borner K, Koeppe P.** 1985. Pharmacokinetics of ciprofloxacin after oral and parenteral administration. Antimicrob. Agents Chemother. **27**:375–379.
8. **Hosaka M, et al.** 1992. In vitro and in vivo antibacterial activities of AM-1155, a new 6-fluoro-8-methoxy quinolone. Antimicrob. Agents Chemother. **36**:2108–2117.
9. **Hsieh MH, Yu CM, Yu VL, Chow JW.** 1993. Synergy assessed by checkerboard. A critical analysis. Diagn. Microbiol. Infect. Dis. **16**:343–349.
10. **Jones RN, Stilwell MG, Hogan PA, Sheehan DJ.** 2007. Activity of linezolid tested against uncommonly isolated gram-positive organisms (3,251 strains): report from the SENTRY Antimicrobial Surveillance Program. Antimicrob. Agents Chemother. **51**:1491–1493.
11. **LaPlante KL, Sakoulas G.** 2009. Evaluating aztreonam and ceftazidime pharmacodynamics with *Escherichia coli* in combination with daptomycin, linezolid, or vancomycin in an in vitro pharmacodynamic model. Antimicrob. Agents Chemother. **53**:4549–4555.
12. **Liu XG, Li RC.** 2001. Effects of repeated rifabutin administration on the pharmacokinetics of intravenous and oral ciprofloxacin in mice. J. Chemother. **13**:563–568.
13. **MacGowan AP.** 2003. Pharmacokinetic and pharmacodynamic profile of linezolid in healthy volunteers and patients with Gram-positive infections. J. Antimicrob. Chemother. **51**(Suppl 2):ii17–25.
14. **Mortin LI, et al.** 2007. Rapid bactericidal activity of daptomycin against methicillin-resistant and methicillin-susceptible Staphylococcus aureus peritonitis in mice as measured with bioluminescent bacteria. Antimicrob. Agents Chemother. **51**:1787–1794.
15. **Odds FC.** 2003. Synergy, antagonism, and what the chequerboard puts between them. J. Antimicrob. Chemother. **52**:1.
16. **Pfizer.** 2012. Zyvox® (linezolid) injection, (linezolid tablets, (linezolid) for oral suspension. Pfizer LAB-0139-21.0, revised February 2012. http://labeling.pfizer.com/ShowLabeling.aspx?id=649.
17. **Sisson TL, Jungbluth GL, Hopkins NK.** 1999. A pharmacokinetic evaluation of concomitant administration of linezolid and aztreonam. J. Clin. Pharmacol. **39**:1277–1282.
18. **Slatter JG, et al.** 2002. Pharmacokinetics, toxicokinetics, distribution, metabolism and excretion of linezolid in mouse, rat and dog. Xenobiotica **32**:907–924.
19. **Stalker DJ, Jungbluth GL.** 2003. Clinical pharmacokinetics of linezolid, a novel oxazolidinone antibacterial. Clin. Pharmacokinet. **42**:1129–1140.
20. **Sweeney MT, Zurenko GE.** 2003. In vitro activity of linezolid combined with other antimicrobial agents against staphylococci, enterococci, pneumococci, and selected gram-negative organisms. Antimicrob. Agents Chemother. **47**:1902–1906.
21. **Thallinger C, et al.** 2008. Effect of severity of sepsis on tissue concentrations of linezolid. J. Antimicrob. Chemother. **61**:173–176.
22. **Wilcox MH, et al.** 2009. Complicated skin and skin-structure infections and catheter-related bloodstream infections: noninferiority of linezolid in a phase 3 study. Clin. Infect. Dis. **48**:203–212.
23. **Yanagihara K, et al.** 2002. Efficacy of linezolid against methicillin-resistant or vancomycin-insensitive *Staphylococcus aureus* in a model of hematogenous pulmonary infection. Antimicrob. Agents Chemother. **46**: 3288–3291.